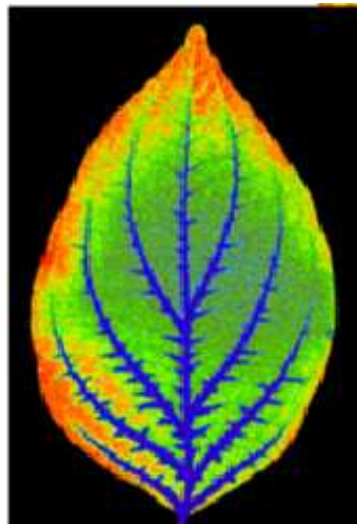




USER MANUAL



Chlorophyll Fluorescence Imaging System

User manual for Technologica *FluorImager* software for use with
Technologica cf Imager hardware

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www.technologica.co.uk

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Before you start

The following information is for instrument use and assumes the user has installed the supplied hardware and software.

The instrument exterior is designed using Dutch-folded panels to virtually exclude all external light, thus preventing background noise influencing the sample or fluorescence measurements. If the instrument is being used in a dark room, all side and front panels can be removed by releasing the four corner screws of each panel, (using the 2mm driver supplied). This feature can also be useful for examining leaves that are still attached. The base of the instrument can be removed for in-situ measurements. The front user access door of the instrument is detachable by gently lifting upwards off its hinges. This allows increased access which can be particularly useful when aligning samples. Cams on the central sample stage enable a variety of well plates to be accurately located. If required the sample stage can be removed from the base plate using the 3mm socket driver supplied.

Your instrument is factory set for light and camera calibrations, that are unique to your instrument, with the data held on the installation CD. In the unlikely event that you need to recalibrate the camera or the lights refer to Appendix I. This may be necessary if the camera is replaced or the lights require readjustment. It is also important to note that the factory default setting refers to a nominal sample stage height of **140 mm** above the base of the instruments (zero on the fixed scale aligned with 30 on the moving scale of the sample stage rack and pinion). This can be locked into position using the small lever at the rear of the stage. It is important to ensure that all measurements are conducted using this **140mm reference height**, as light intensity, alignment and area measurements are all calibrated to this height. Because measurements are referenced to this nominal height, the sample stage must be lowered by the mean height of the sample.

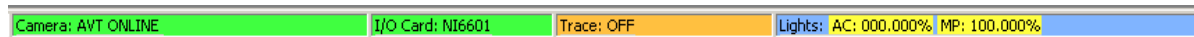
The FluorImager software is designed to be as user friendly as possible. Several new features and modifications have been included in the new version of the software (Version 2.2). Below we have outlined several new features that help users identify images and options.

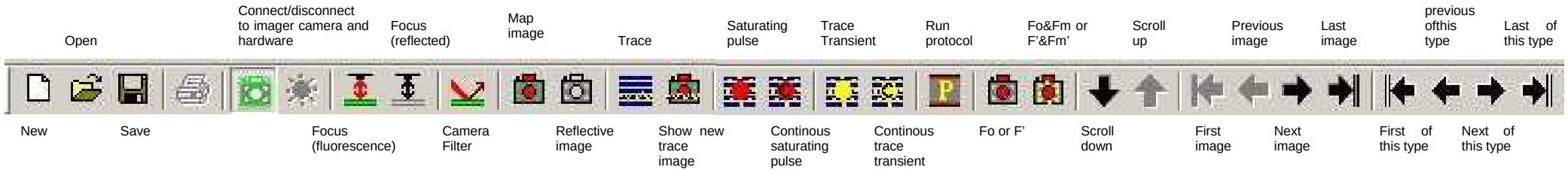
- 1) Thumbnail map and regions images are given a colour bar at the top of the image which corresponds to the colour traces.
- 2) To assist identification of image types, all thumbnail labels are in different colours according to the image type
 - a. Map image – white
 - b. Regions – grey
 - c. *Fo* – red
 - d. *Fm* – blue
 - e. *Fv/Fm* – Cyan
 - f. *Fq'/Fm'* – turquoise
 - g. NPQ - yellow
 - h. *Fv'/Fm'* – dull yellow
 - i. *Fq'/Fv'* – yellow/green
- 3) When the mouse is moved over a menu bar key the button is highlighted. The button will change colour if it has been selected (and is a permanent selection)

and depressed if it is a transient selection. When the cursor is held over a button, a display menu appears showing the name of the button (for further details on the button bar below).



- 4) A status tool bar has been added to the bottom of the screen. Using colour coding, this bar indicates; 1) whether the camera is connected, 2) the identification of an I/O card, 3) whether the trace is running or not, 4) the settings of actinic and measuring pulse lights, and 5) whether the machine needs calibrating (a display will appear in the far right of the status bar).





It is worth noting that not all menu bar button options are available at any one, but are determined by the user options. For example 'Trace button' options are not available if a trace is not running.

Main Menus

The main File, Window and Help menus are standard windows menus and need no further explanation here.

Edit Main Menu Items

Cut (Delete) image	Ctrl+X
Copy image	Ctrl+C
Paste image	Ctrl+V
Copy selected images	Ctrl+F
Paste selected images	Ctrl+H
Copy active pixels	Ctrl+E
Paste active pixels	Ctrl+I
Copy&Paste active pixels to all	Ctrl+A
Cut selection size	Shift+Ctrl+X
Copy selection size	Shift+Ctrl+C
Paste selection size	Shift+Ctrl+V
Copy&Paste selection size to all	Shift+Ctrl+A
Copy palette settings	Shift+Alt+C
Paste palette settings	Shift+Alt+V
Copy&Paste palette settings to all	Shift+Alt+A
Copy data limits	Ctrl+Alt+C
Paste data limits	Ctrl+Alt+V
Copy&Paste data limits to all	Ctrl+Alt+A
Cut zone lines	
Copy zone lines	
Paste zone lines	
Copy&Paste zone lines to all	
Copy to Clipboard	

Edit → cut (Delete) image, Copy image and Paste image

Cut, Copy and Paste the [current image](#). Images can be copied and/or moved from one location to another within the same file or from one file to another.

Edit → copy selected images and paste selected images.

Multiple images can be copied and pasted to another location, within the same file or to a different file. Multiple images are selected from the [thumbnail images](#), by clicking the left mouse button while holding down the Ctrl key. Images are pasted in the order that they originally occurred within the file they were copied from, not the order that they were selected in.

Edit → copy and paste active pixels and copy & paste active pixels to all.

The active pixel locations can be copied from one image and pasted to another or to all images. One instance where this can be useful is when a second file is started with the same sample. This command allows the active pixel locations within the [map image](#) of the first file to be copied to the map image of the second file. These commands can also be useful when [region images](#) are being used to divide a sample.

Edit → Cut selection size, Copy selection size, Paste selection size and Paste selection size to all

A rectangular selection can be made from within an image by clicking the left mouse button at one corner of the required area and dragging to the opposite corner, while

holding down the Shift and Ctrl keys. The coordinates of this selection can be copied and pasted to other images in the same file or a different file.

Edit → Copy and Paste palette settings and Copy & paste palette settings to all.

The palette settings for one image can be copied from one image and pasted to another.

Edit → Copy and paste data limits and copy & paste data limits to all.

Allows users to copy palette settings from one image and paste them to another or all other images. This is particularly important if selected images are being compared with each other using the same colour palette scale.

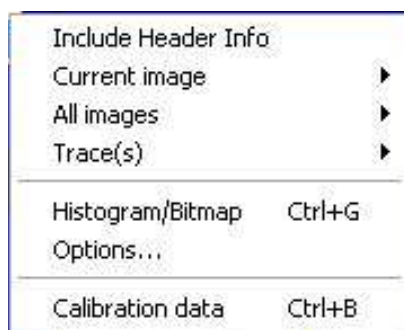
Edit → Cut zone lines and copy zone lines & paste zone lines and copy & paste zone lines to all.

Zone lines can be copied from one image to another or pasted to all images. Using the cut command removes all zone lines from the selected image.

Edit → Copy to clipboard....

The extensive range of clipboard copy functions are described below.

Edit→Clipboard copy



Edit → Clipboard copy → Include Header Info.

With this box checked, details including user name, image information, comments and experimental details are copied along with the data to the clipboard.

Edit → Clipboard copy → Current image →, All images → and Trace(s) →

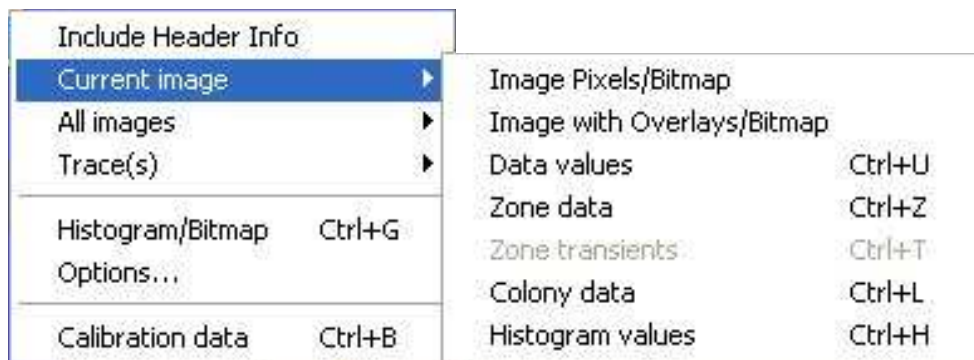
Leads to sub-menus for copying the [current image](#) and related data, data from all images and trace(s) and related data.

Edit → Clipboard copy Histograms and Histogram options...

The histograms associated with the [current image](#) can be copied in various ways.

Edit → Clipboard copy → Calibration data

The calibration data option is normally greyed out; the calibration data are only meaningful to trained technical support.

Edit→Clipboard copy→Current image

Edit → Copy to clipboard → current image → Image pixels/Bitmap & Image with overlays/Bitmap.
The [current image](#) can be exported as a Windows bitmap (to programs such as PowerPoint). If an area of the image has been selected, only this area is copied.

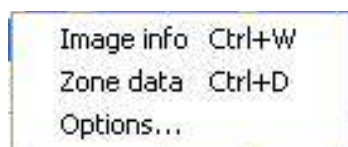
Edit → Copy to clipboard → current image → Data values.
The image can be copied as an array of pixel data values, which can be pasted into spreadsheet applications, or to other data analysis applications.

Edit → Copy to clipboard → current image → Zone data.
The data from each zone in an image can be copied and pasted into Excel (or similar). This includes the active area within each zone, the mean fluorescence parameter value and, with transient images; 1 – I/P, 1 – O/P, 1 – O/I and the area between O and P. If zones have not been identified within the current image, mean values of area and the fluorescence parameter for the particular image will be copied.

Edit → Copy to clipboard → current image → Zone Transients.
When a transient image is selected as the [current image](#), there is an option to copy the transient points from each zone, for pasting into Excel and other spreadsheet-based applications.

Edit → Copy to clipboard → current image → Colony data.
Similar to zone data, colony data from the currently selected image are copied to the clipboard. These data include colony number, x,y position, area and the specific fluorescence parameter for all identified colonies within an image. If no colonies have been selected, image mean values for each data category will be copied.

Edit → Copy to clipboard → current image → Histogram values.
The values from the data and palette histograms are both copied, using this command.

Edit→Clipboard copy→All images

Edit → Clipboard copy → All images → image info

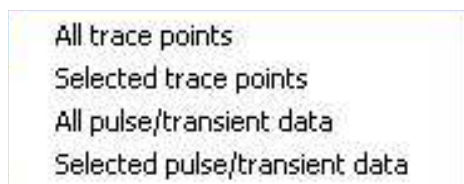
This command creates a table of data from all images in the current file, which can be pasted into Excel and other spreadsheet-based applications.

Edit → Clipboard copy → All images → Zone data

This command creates a table of zone data from all images in the current file, which can be pasted into Excel and other spreadsheet-based applications.

Edit → Clipboard copy → All images → Options...

This launches the Images data clipboard options dialogue, which provides options relevant to the copying of whole image data and zone data.

Edit→Clipboard copy→Trace(s)Edit → Clipboard copy → Trace(s) → All trace points

This command creates a table of data from all trace points in the current file, which can be pasted into Excel and other spreadsheet-based applications.

Edit → Clipboard copy → Trace(s) → Selected trace points

This command creates a table of data from selected trace points in the current file, which can be pasted into Excel and other spreadsheet-based applications. Trace points can be selected by left mouse click and dragging the cursor over the trace area to be selected whilst holding down the shift and ctrl keys. A blue boarder on the trace will highlight the area that has been selected.

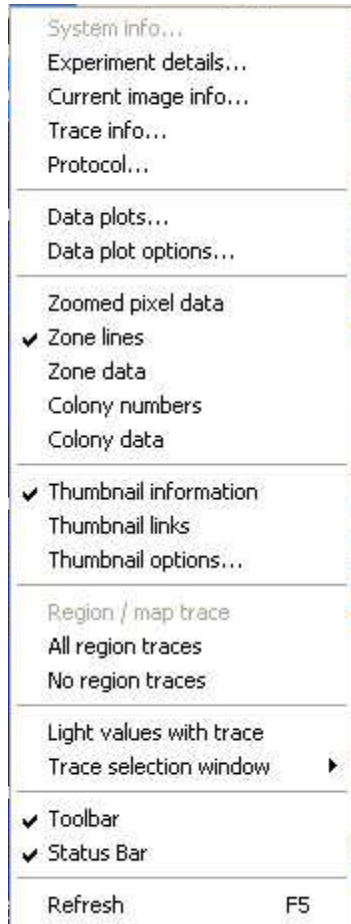
Edit → Clipboard copy → Trace(s) → All pulse/transient data

This command creates a table of data from all trace points for all regions in the current file, which can be pasted into Excel and other spreadsheet-based applications. With Include header info selected, all experimental details and trace comments are copied along with raw fluorescence. With Include header info not selected, the data and comments are copied but without the experimental and user information. This menu can also be accessed by right clicking the mouse on the main fluorescence trace which opens up a dialogue box in which the user can select various trace copy options.

Edit → Clipboard copy → Trace(s) → selected pulse/transient data

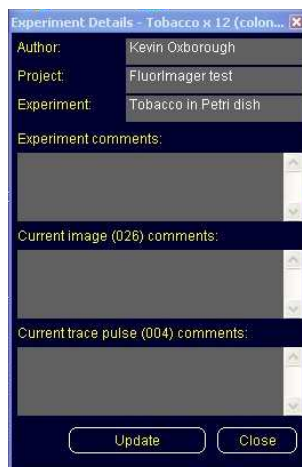
Creates a table of data from all selected pulses/transient data for all regions in the within the current file, which can be pasted into Excel and other spreadsheet-based applications. Selections are made by either clicking on the pulse/transient of interest, which will be highlighted with a blue triangle for a pulse or yellow triangle for a transient). Alternatively, an area of a trace can be highlighted by hold ctrl + shift and dragging the mouse over the area of the trace you wish to copy, (the area will be highlighted with blue boarder lines on the top and bottom of the trace window). With Include header info selected, all experimental details and trace comments are copied along with the fluorescence parameters. With Include header info not selected, the data and comments are copied but without the experimental and user information.

View Main Menu Items



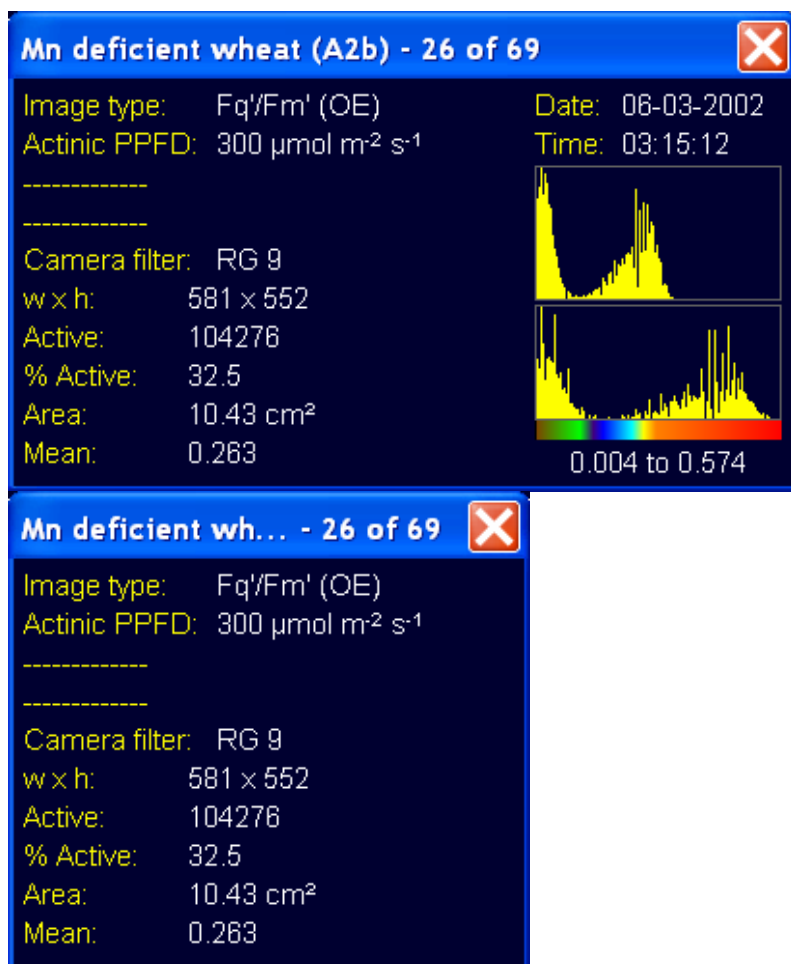
View → Experimental details...

Opens up Experimental details dialogue where experimental information such as author, project, experiment and comments can be included. This dialogue box allows input of [current image](#) and current trace pulse comments which can be copied with the data to a spreadsheet.



View → Current image info...

Accessed through View→Current image info... or by selecting Image info from the popup that is launched when the right mouse button is clicked within the main image. This dialogue provides information about the [current image](#). It is a 'modeless' dialogue, which means that other parts of the program can be accessed while it is open. It can be switched between its larger and smaller formats by selecting Show more and Show less, respectively, from the popup menu that is launched when the right mouse button is clicked. The dialogue can be hidden by selecting Hide window from the same popup menu. If this is done, the dialogue will appear in its last location and size format when accessed again, rather than the default condition.



View → Trace info...

Accessed through View→Trace info... or by selecting Trace info from the popup that is launched when the right mouse button is clicked within the trace window. This dialogue provides information about the [Fm](#) or [Fm'](#) pulse that is currently selected. It is a 'modeless' dialog, which means that other parts of the program can be accessed while it is open.

Mn wheat (F3) - Pulse 9 of 14 (linked to 1)

Trace time:	00:22:34 s	NPQ:	0.65 (0.77)
Actinic PPFD:	300 $\mu\text{mol m}^{-2} \text{s}^{-1}$	PSII flux:	53.7 (63.0)
Pulse PPFD:	2750 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Fq'/Fm':	0.18 (0.21)
Pulse length:	1000 ms	Fv'/Fm':	0.43 (0.47)
FPSI:	20.0% of Fo	Fq'/Fv':	0.42 (0.45)
Camera filter:	RG 9		

The dialogue can be hidden by selecting Hide window from the popup menu that is launched when the right button is clicked. If this is done, the dialogue will appear in its last location when accessed again, rather than the default location. The dialogue will be updated with the selection of a different pulse from the trace (left click on trace pulse). If the current level of PSI fluorescence (FPSI) is assumed to be greater than zero (see the PSI fluorescence dialog), the value of all parameters is calculated assuming both zero PSI fluorescence and the selected value of PSI fluorescence; the latter value being shown in brackets.

View → Protocol...

Opens current protocol dialogue window. A protocols represent the highest level of automation within the FluorImager program. A protocol allows the user to set up an automated procedure that can be saved and retrieved. A user can change light intensity, apply pulses, apply transients and auto pulses, all of which can be timed exactly. There is also an option to repeat various steps within a protocol using different cycle numbers. Commands within a protocol can be altered, updated, copied and pasted.

Protocol

After a delay of ☒ min ☐ s

Perform the following action:

☒ Change actinic ☐ Apply pulse

☐ Apply transient ☐ Auto pulse

This number of times

Details: % max.

Pulse PPFD

Pulse length (ms)

☒ Take F' & Fm' images with pulses

☒ Only save images on the last pulse

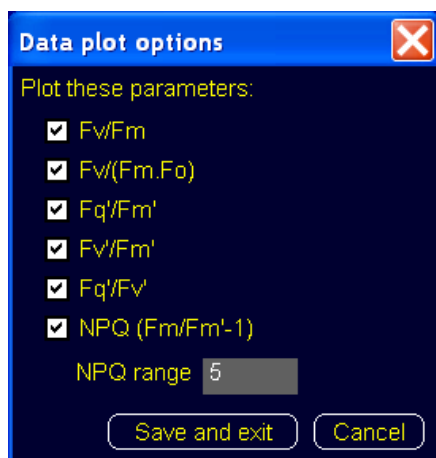
Step	Delay	Action	Cycles	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
1	5 s	Apply pulse	1	4000
2	5 s	Change actinic		50
3	5 min	Apply pulse	1	4000
4	5 s	Change actinic		100
5	5 min	Apply pulse	1	4000
6	5 s	Change actinic		150
7	5 min	Apply pulse	1	4000
8	5 s	Change actinic		200
9	5 min	Apply pulse	1	4000
10	5 s	Change actinic		300
11	5 min	Apply pulse	1	4000
12	5 s	Change actinic		400
13	5 min	Apply pulse	1	4000
14	5 s	Change actinic		500
15	5 min	Apply pulse	1	4000
16	5 s	Change actinic		600
17	5 min	Apply pulse	1	4000
18	5 s	Change actinic		800
19	5 min	Apply pulse	1	4000
20	5 s	Change actinic		1000
21	5 min	Apply pulse	1	4000
22	5 s	Change actinic		0
23	5 min	Apply pulse	3	4000

Step 23 of 23 Total time: 01:06:00 s Time remaining: 00:00:00 s

View → Data plots and Data plot options....



This dialogue plots up to six fluorescence parameters, calculated from the fluorescence trace data. It can be shown with or without a key on the right hand side. The default location for this dialogue is directly above the fluorescence trace area of the main window. This is a modeless dialogue, which means it can remain open while other parts of the program are being accessed. It is updated dynamically, which means that parameter values are calculated and shown immediately after the application of a saturating pulse and are updated if the assumed level of PS I fluorescence is changed (see PS I fluorescence dialog). The parameters that are shown can be set through the Data plot options dialogue.



Accessed through View→Data plot options... or by selecting Data plot options... from the popup that is launched when the right mouse button is clicked within the Data plots dialogue. Changes to settings are made when the Save and exit button is pressed.

View → Zoomed pixel data, Zone lines, Zone data, Colony numbers and Colony data.

Each of these options can be displayed on the current image if selected. The zoomed pixel data is only shown when the zoom has been applied to the current image.

View → Thumbnail information, Thumbnail links and Thumbnail options.

These options can be selected to on and off to allow information such as actinic light level, filter and time to be displayed on each of the thumbnails. The thumbnails used to build images can be displayed using the Thumbnail links option. The types of thumbnails displayed can be selected in Thumbnail options.

View → Region/map trace, All region traces and No region traces.

Option which allows the user to define which traces are displayed. If Region/map trace is ticked and No region traces is selected only the map trace will be shown. If Region/map is not ticked no traces will be displayed. All region traces displays all traces with and without the map image.

View → Light values with trace

Selection of this view option add an additional light values trace to the trace display.

View → Trace selection window...

Options within this menu allow either Last 30 s of a trace to be displayed in the selection window, or the current section or the selected saturating pulse.

View → Toolbar and Status Bar and Refresh

Toggles between on and off for the display of the Toolbar (top of the screen) and Status Bar (bottom of the screen). New changes to the view display can be viewed using Refresh or F5.

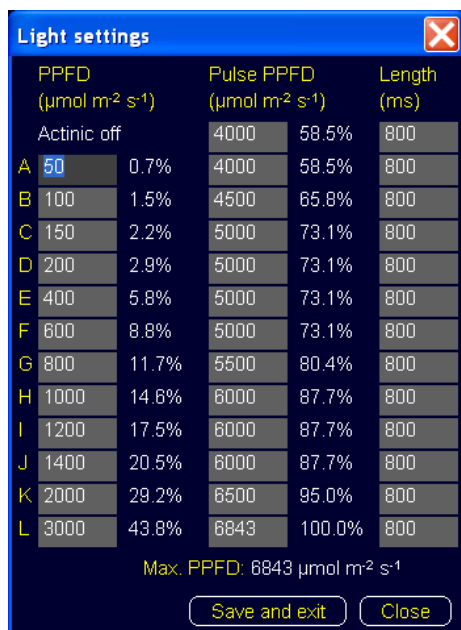
Settings Main Menu Items



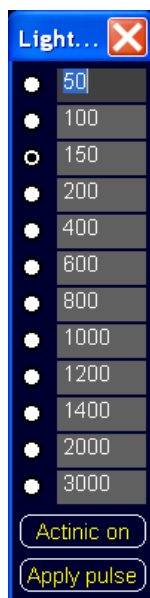
Settings → Lights...

Allows manual setting of the light levels, and manual control of light level changes.

If the FluorImager programme is connected to the camera, the light setting dialogue is launched when Settings → Lights (Alt+L) is selected. In addition to zero light, twelve different stock [PPFDs](#) can be set (A to L), which are used when the data is being entered in manual mode. All the user has to do is to enter the desired [PPFD](#) in the PPFD column (as an integer value between 0 and the maximum actinic PPFD). The FluorImager program calculates the duty cycle for the requested value, which is given as a percentage of the maximum level (%max.). If the precise [PPFD](#) value cannot be provided, the program updates the requested value with the nearest available value (this will normally be within $1 \mu\text{mol m}^{-2} \text{s}^{-1}$ of the requested value). This is a 'modal' dialogue, which must be closed before the user can interact with the rest of the program.

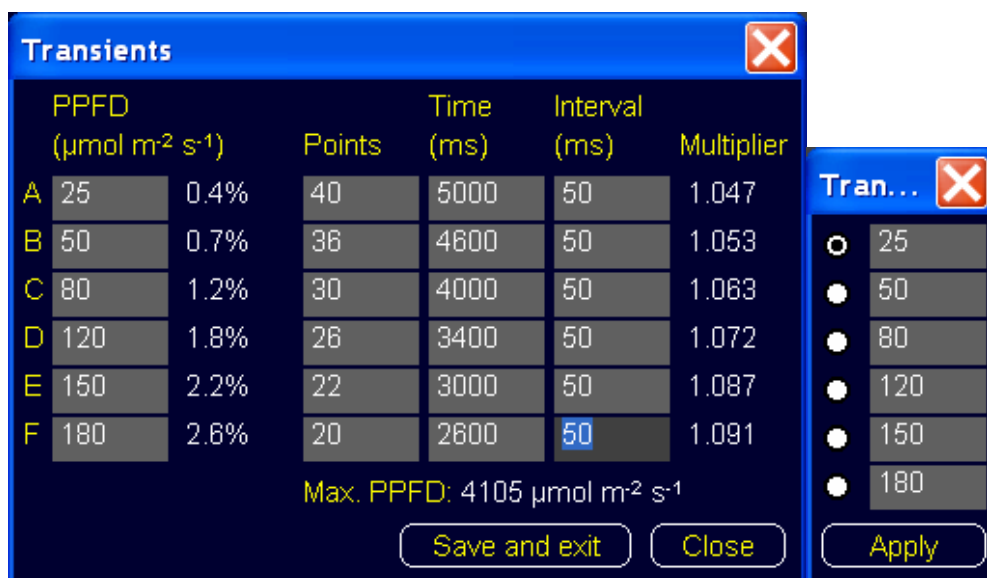


When the program is connected to the camera, the Light dialogue (below) is launched. This is a '[modeless](#)' version, which can be left open while the user interacts with the rest of the program. This version of the dialogue can be 'shrunk' by clicking the right mouse button within the background of the dialogue and selecting Just show actinic PPFDs. It can be expanded again by clicking the right mouse button and selecting Show pulse details. When the Actinic on button is pressed, it turns Red-Orange until it is pressed again (to switch the light off). When the Apply pulse button is pressed, the button turns Red-Orange for the duration of the pulse.



Settings → Transients...

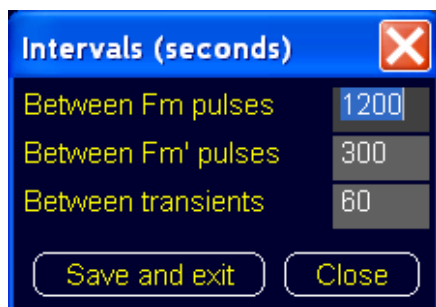
Reached through Settings/Transients... (Alt+T), it allows for the setting up of six stock transient protocols (A – F).



The user sets the incident [PPFD](#) during the [transient](#). The *FluorImager* program calculates the duty cycle for the requested value, which is given as a percentage of the maximum level (%max.). The user also sets the number of Points, Time (ms) and the initial Interval (ms) between points. The *FluorImager* program calculates the Multiplier value that is required to satisfy these settings. Illegal settings are corrected automatically. For example, if the user sets Points to 30 when Time is 1000, Time is increased to 1500 (the minimum time that 30 points can be taken in). If Time is then shortened back to 1000, Points is decreased to 20 (the maximum number of points that can be taken in this time). The 'O' fluorescence level (same as [Fo](#)) is recorded before the light is applied, while the 'I' fluorescence level is taken as the first point during the transient. The program automatically searches the remaining trace points to find the peak (P). If the *FluorImager* program is not connected to the camera, the left most dialogue (above) is launched when Settings → Transients (Alt+T) is selected. This is a 'modal' dialogue, which must be closed before the user can interact with the rest of the program. When the program is connected to the camera, the middle dialogue is launched. This is a 'modeless' version, which can be left open while the user interacts with the rest of the program. This version of the dialogue can be 'shrunk' by clicking the right mouse button within the background of the dialogue and selecting Just show PPFDs. It can be expanded again by clicking the right mouse button and selecting Show details. When the Apply transient button is pressed, the button turns Red-Orange for the duration of the transient.

[Settings](#) → Intervals...

Accessed through Settings/Intervals... (Alt+S), the values set here define the time intervals between [saturation pulses](#) in the dark and light adapted states and between [transients](#). These values are used when continuous pulses and/or continuous transients are applied.



Settings → Protocol...

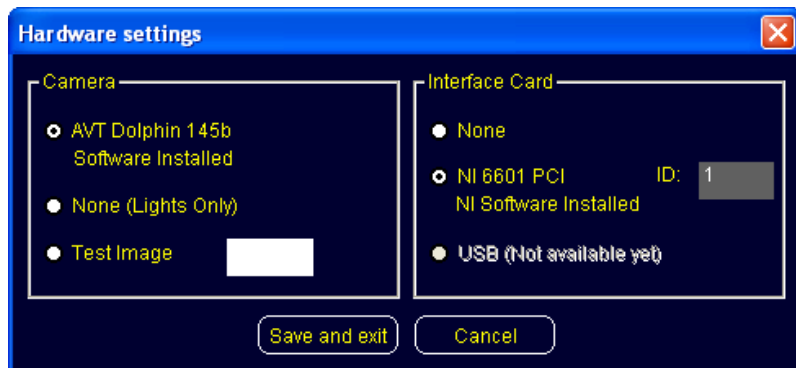
Opens the [protocol](#) dialogue window allowing users to define an experimental protocol. The protocol may include changing actinic light level, applying a [saturating pulse](#), applying a transient or auto pulse. These options can all be set with precise timing and repeated in cycles.

Settings → .pcl (protocol files)...and .set (settings) files...

Users can save and reload [protocols](#) and [settings files](#) and the default settings file can be restored using Settings → .set (settings) files → load defaults.

Settings → Hardware

These are factor set for camera hardware and interface and should not be altered by the user.

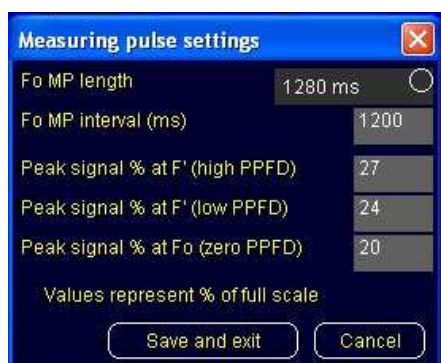


Settings → Camera filters ...

Allows input of filter names. Not applicable with current system.

Settings → Measuring pulses ...

Sets measuring pulse length, interval, and % peak signal..



Settings → Calibration ...

Camera and light calibration. This is factory set, but should users wish to re-calibrate the instrument see Appendix I.

Image Main Menu Items



Image(s) → Details...

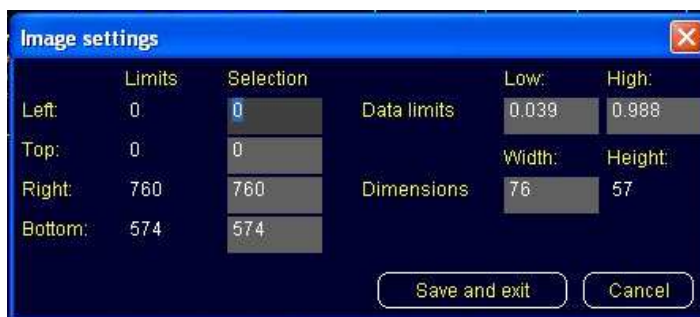
Launches the Image details dialogue.



The image details dialogue launches from the [thumbnail](#) that is selected at that time. The type of image can be changed using the wheel (top corner of image type text box). If copy is ticked the copy location will be identified. The camera filter (if appropriate) and user are also displayed. A text box allows comments to be included and saved for that image type.

Image(s) → Settings...

Launches the Image settings dialogue.



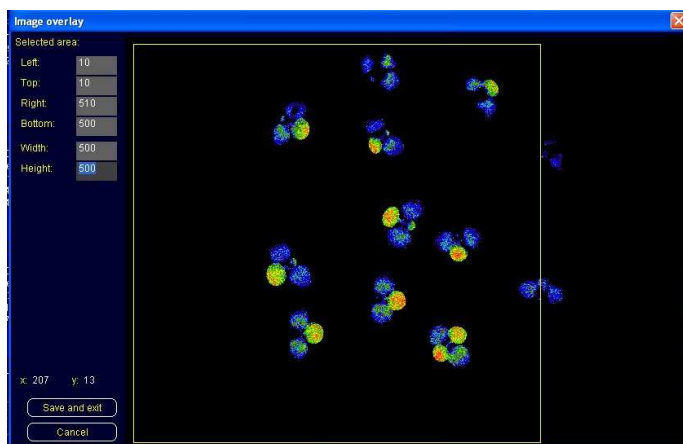
Allows user to input selection area and dimensions. The most useful parameter is the data limits, allowing the user to define the low and high limits, e.g. a series of images can all be displayed using an identical defined colour scale.

Image(s) → Modify...

Launches the Modify image dialogue (see How To Guide for information).

Image(s) → Edit overlay...

Launches the Edit overlay dialogue



Edit overlay allows manual numerical selection of the area of the image. Moving x,y co-ordinates give the position of the cursor within the image.

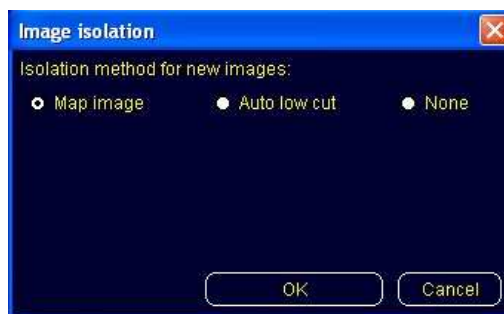
Image(s) → Horizontal and vertical flip....
Flips map image Horizontal/Vertical.

Image(s) → Apply isolation & Undo isolation

After a map image has been taken, the Apply isolation command can be used as an automated method for isolating the active area(s) within the image. If the isolation was unsuccessful (if required pixels were deleted by the automated process) the isolation can be reversed using the Undo isolation command. A manual isolation can then be performed using the tools provided within the Modify image dialogue. The Image isolation options... command launches the Isolation options dialogue.

Image(s) → Isolation options...

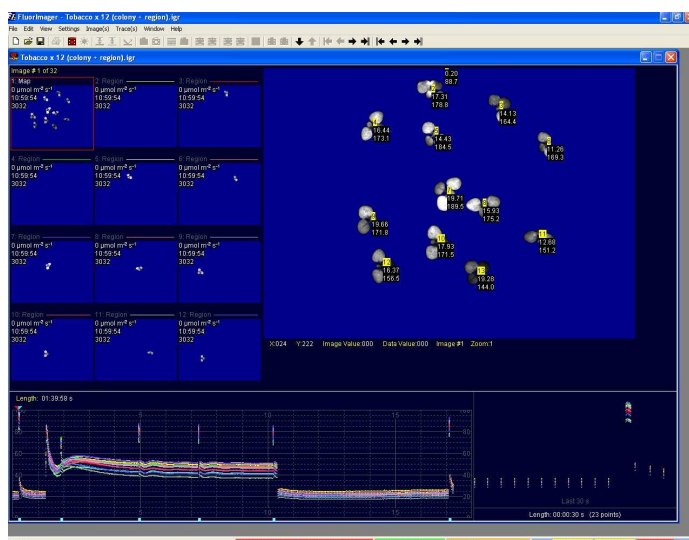
If Map image is selected, pixels within new images are only active if their value is greater than zero within both the newly acquired data array and the map image. If Auto low cut is selected, the program increases the value of a low pass filter until the number of active pixels within the newly acquired data array is the same as in the map image.



If None is selected, no isolation method is applied. The Map image option is best for samples that don't move during the period of imaging. The Auto low cut option is useful in situations where some sample movement is likely.

Image(s) → Auto region images

Runs the automated region image function. This creates a region image for each isolated area within the map image. This function can only be performed after a map image has been taken.



Auto region images isolated area within the map image, labels them and automatically creates regions for each area. Each region is displayed as a thumbnail after the map image in the order they were created. If run trace is selected, a trace will be displayed for each region. The areas are numbered according to their position in the current image (from top left to bottom right).

At present only 14 separate regions can be created; if there are more than 14 regions the following warning will appear. Regions will be created for the first 14 isolated areas and although the remaining area will be numbered and identified, regions will not be created.



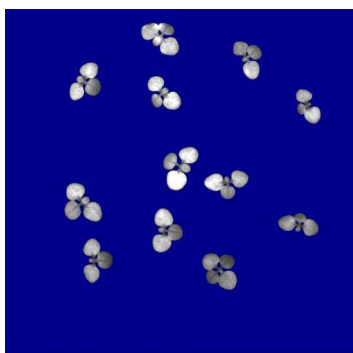
Image(s) → Boolean operators →

A AND B
A OR B
A XOR B
A NOT B

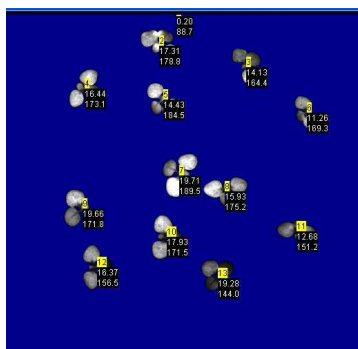
These commands can be used in the construction of [region images](#). Both the A and B images (which can be the [map image](#) plus a region image or two region images) are selected from the thumbnail region. First, the 'A' image is selected. Next, the 'B' image is selected while holding down the Ctrl key. Finally, the required operator is selected from the menu. The resultant image is inserted after the 'B' image.

Image(s) → Isolate colonies & Merge colonies

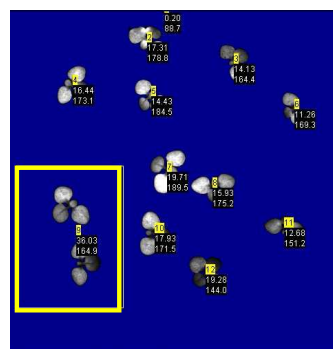
The Isolate colonies command isolates up to 250 [colonies](#) within the map image. The Merge colonies command can be used to merge two or more isolated colonies.



Map image before colony isolation.



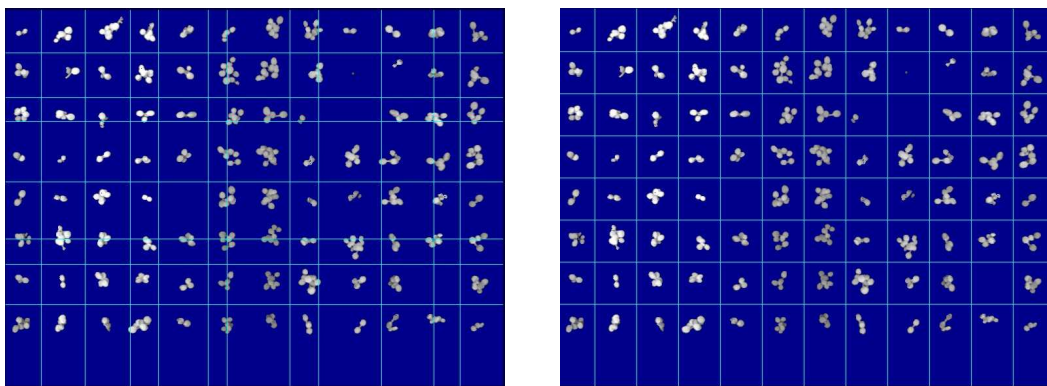
Colony isolation applied. All separate colonies are numbered and labelled starting at the top left.



Two colonies in yellow box have been merged. Note, now there is only one number and label for the two colonies.

Image(s) → Auto-space zone lines

This command automatically distributes both horizontal and vertical zone lines evenly across the image.



The left image shows uneven distribution of horizontal and vertical zone lines over a 96 well plate. After the application of Auto-space zone lines, the horizontal and vertical zone lines are even distributed in both directions (right image).

Image(s) → Select as the [current Fm](#)

If the [current image](#) or trace saturation pulse was taken in the dark, this command can be used to make it the [current Fm](#) image or pulse. The [current Fm](#) image will be used to calculate all other fluorescence parameter based upon dark adapted [Fm](#). The position of the [current Fm](#) image on the trace is indicated by a red triangle.

Image(s) → Smooth & Smoothing of new [Fo](#) images →

If the chlorophyll fluorescence signal from a sample is weak (possibly due to a low concentration of chlorophyll within the sample), the noise within the image can be significant. This is particularly true with [Fo](#) images. If noisy [raw images](#) are used in the construction of [parameter images](#), the spread of values within the parameter image will be much greater than the true situation. The Smoothing commands can be used to apply a smoothing algorithm to noisy raw images. This algorithm has been written in such a way that it has minimal effect on the overall mean value of the image. When a smoothing routine is applied to a non-smoothed image, a copy of the image is automatically made. No copy is made if a smoothing routine is applied to an image that has already been smoothed at least once.

Image(s) → Crop to selection boundaries & Crop all to selection boundaries

These commands crop the [current image](#), or all images within the file, to the coordinates of the current selection.

Image(s) → Restore map

When a [map image](#) is taken, a backup is automatically made. This image is stored when the file is saved to disk. If live pixels are subsequently deleted, using the tools in the [Modify image dialog](#), The Restore map command can be used to revert to the original map image.

Image(s) → Maximise image area

The Maximise image area command increases the size limits of the image to the maximum size possible (see Image settings dialog) and assigns a value of 128 to every pixel. This option is no longer necessary for current software.

Image(s) → Build →

The options that are available depend upon what type of image is current.

If the [current image](#) is an [Fm](#) image, the available options are:

Fv/Fm
Fv/(Fm.Fo)
Fq'/Fm'
Fv'/Fm' & Fq'/Fv'
NPQ (Fm/Fm' - 1)

If the current image is an [Fm'](#) image, the available options are:

Fv/Fm
Fv/(Fm.Fo)
Fq'/Fm'
Fv'/Fm' & Fq'/Fv'
NPQ (Fm/Fm' - 1)

No options are available with other image types. Appropriate build options also appear on the popup menu that is launched if the left mouse button is pressed when the cursor is within the current image area.

Image(s) → Auto build options...

Launches the Auto image build options dialog.



Checking a box allows the user to define the images that are built with each saturating pulse. The construction of certain images will depend upon particular parameter images being available. E.g. it is impossible to build an image of [NPQ](#) without a dark adapted [Fm](#) image.

Image(s) → Zoom on/off

Applies to the [current image](#). If this is checked, the selected area of the image is displayed at the highest magnification that fits within the image area.

Trace Main Menu Items



Trace(s) → take images with pulses

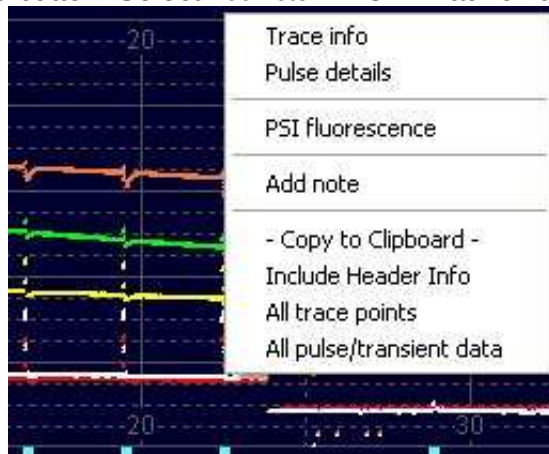
With this item selected images of selected parameters (established in auto build option) will be automatically built and placed as thumbnails with each saturating pulse.

Trace(s) → add note, edit note & delete note

This enables the user to add specific notes to the trace at any time points specified by the user in hours, minute and seconds: Enter the time into the appropriate boxes and comment in the text box below. When save and exit is pressed, the position of the comment is indicated by a green square, located above the trace at the point of insertion. Added comments are copied with the data into spreadsheets.



Trace comments can also be included directly on the trace using the mouse cursor. Move the mouse cursor to the point at which a comment is to be added and click the right mouse button. Select Add note. This will launch the Trace note dialog box.



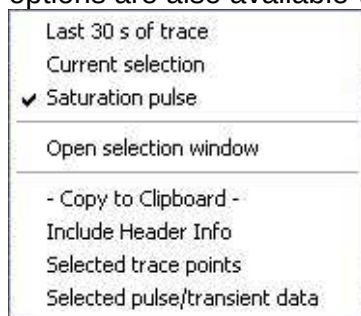
Added comment(s) can be edited or deleted using the main menu edit note/delete note and the correct time being entered into the trace time box. The same menu can also be accessed by a right mouse click on the trace menu at the position of the note to be changed or deleted.

Trace(s) → Open selection window

Opens the trace selection window (box on right side of main trace). The open window can be enlarged using the mouse with drag and drop.

Three options are available for data displayed in the selection window. These options can be accessed using right mouse click in the closed selection window. These are not

accessible when the selection window has been opened. Several copies to clipboard options are also available within this popup menu.

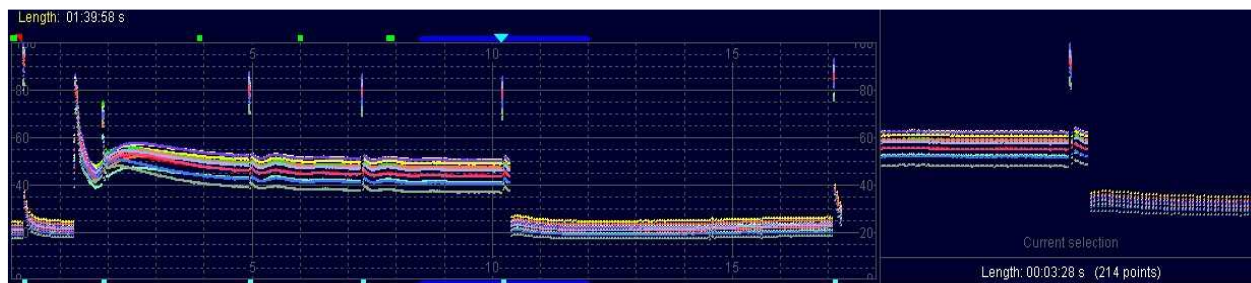


Option 1 – last 30 s of trace

Displays the last 30 s of the current trace, this will be updated as the trace is running.

Option 2 – current selection

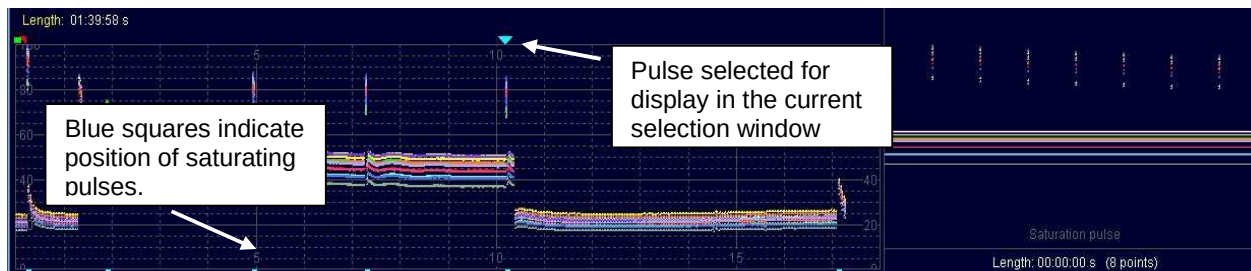
Displays a user defined area. The area can be selected holding the ctrl + shift key down whilst dragging the mouse across the trace area of interest. The selected area is shown on the trace by blue borders.



The selected area (shown by the blue boards on the trace window) is shown in the current selection box at the right of the main trace area.

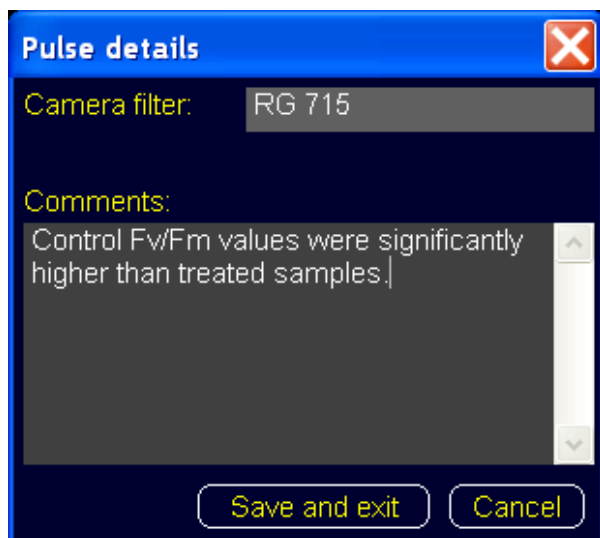
Option 3 – saturating pulse

Displays the last [saturating pulse](#). This will be updated as the trace is running. Particular saturating pulses can be user defined by clicking the mouse on the pulse of interest in the main trace window. The selected pulse is shown by a downward arrow at the top of the trace. The position of saturating pulses within the trace is illustrated by blue squares.



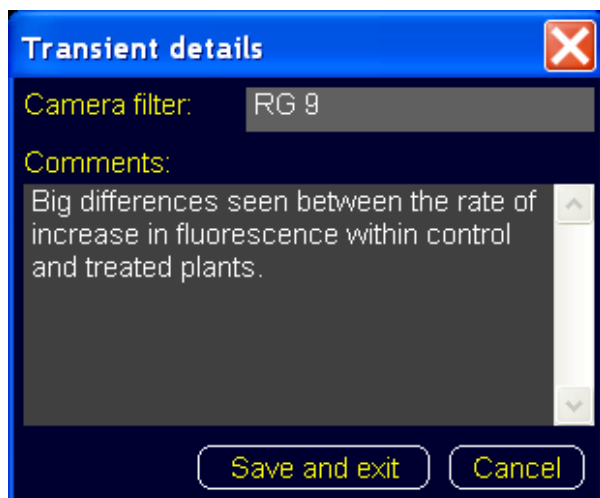
Trace(s) → pulse details...

Accessed through Trace(s) → Pulse details... this dialog allows for the camera filter name to be changed and comments that are specific to the pulse to be edited.



Trace(s) → transient details...

Accessed through Trace(s) → Transient details... This dialogue allows for the camera filter name to be changed and comments that are specific to the [transient](#) to be edited.



Trace(s) → PSI fluorescence....

The dialogue allows the input of the contribution of PSI fluorescence to the total fluorescence signal. Expressed as a percentage of the total fluorescence signal at [Fo](#). If this is unknown FluorImager will assume a value of zero. It is not necessary to complete this box.

Sub Menus

Sub menus can be found by right clicking the mouse button in different regions of the screen. Many of these commands can also be found under the main menus, and are made available as popup menus for convenience. So far we have covered the Modify image menu, and the Trace windows menus above.

Thumbnail Right Click Popup Menu Items



View information, View links and Thumbnail options.

These options are identical to those found under View → Thumbnail information, Thumbnail links and Thumbnail options. View information can be selected to on and off to allow information such as actinic light level, filter and time to be displayed on each of the thumbnails. The thumbnails used to build images can be displayed using the View links. The types of thumbnails displayed can be selected in Thumbnail options.

Cut (Delete) image, Copy image and Paste image

These options are identical to Edit → cut (Delete) image, Copy image and Paste image found under the main **Edit menu**. The options allow the thumbnail images to be Cut, Copied and Pasted from one location to another within the same file or from one file to another.

Copy selected images and paste selected images.

Identical to Edit → copy selected images and paste selected images.

Multiple images can be copied and pasted to another location, within the same file or to a different file. Multiple images are selected from the thumbnail images, by clicking the left mouse button while holding down the Ctrl key. Images are pasted in the order that they originally occurred within the file they were copied from, not the order that they were selected in.

Copy all image info to clipboard

This command is identical to Edit → Clipboard copy → All images → image info

This option creates a table of data from all images in the current file, which can be pasted into Excel and other spreadsheet-based applications.

Current Image Right Click Popup Menu Items

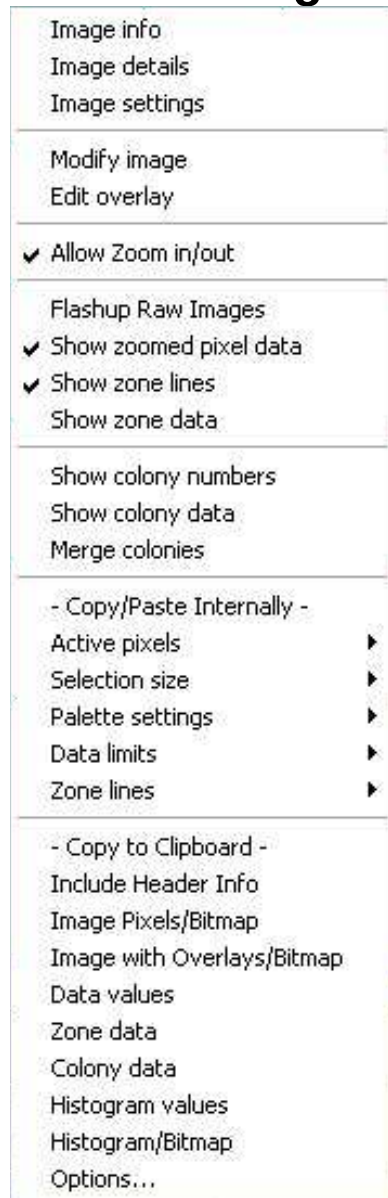


Image Info,

Also found under View(s) → Current image info.

This dialogue provides information about the current image. It is a 'modeless' dialogue, which means that other parts of the program can be accessed while it is open. It can be switched between its larger and smaller formats by selecting Show more and Show less, respectively, from the popup menu that is launched when the right mouse button is clicked. The dialogue can be hidden by selecting Hide window from the same popup menu. If this is done, the dialogue will appear in its last location and size format when accessed again, rather than the default condition.

Image details,

Also found under Image(s) → Details

Launches the Image details dialogue from the current image. The type of image can be changed using the wheel (top corner of image type text box). If copy is ticked the copy

location will be identified. The camera filter (if appropriate) and user are also displayed. A text box allows comments to be included and saved for that image type.

Image setting

Also found under Image(s) → Settings

Launches the Image settings dialogue.

Allows user to input selection area and dimensions. The most useful parameter is the data limits, allowing the user to define the low and high limits, e.g. a series of images can all be displayed using an identical defined colour scale.

Modify image

Modify image opens, for further information see How To Guide.

Edit overlay

Also available through Image(s) → Edit overlay...

Edit overlay allows manual numerical selection of the area of the image. Moving x,y co-ordinates give the position of the cursor within the image.

Allow Zoom on/off

Also found through Image(s) → Zoom on/off

If this is checked, the selected area of the image is displayed at the highest magnification that fits within the image area.

Show zoomed pixel data, show zone lines, show zone data, show colony numbers, show colony data.

Also accessed through View → Zoomed pixel data, Zone lines, Zone data, Colony numbers and Colony data. Each of these options can be displayed on the current image if selected. The zoomed pixel data is only shown when the zoom has been applied to the current image.

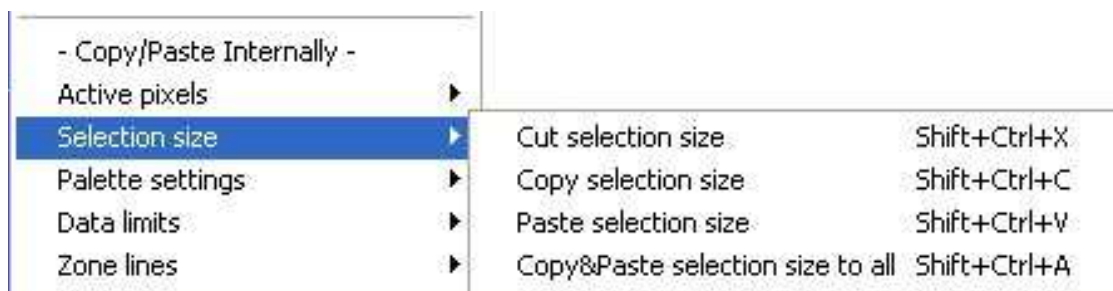
Merge colonies

Found through Image(s) → Merge colonies

Merge colonies can be used to merge two or more isolated colonies.

Copy/Paste Internally

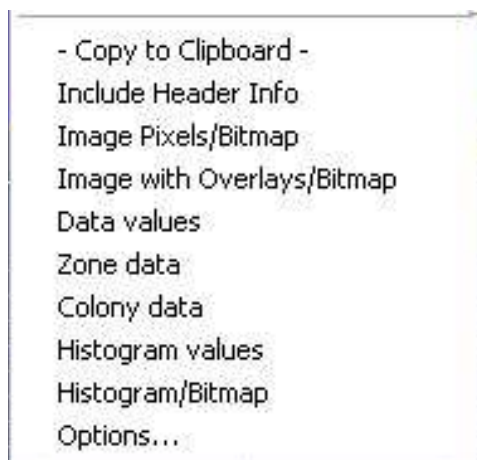
This menu gives the user several options of copy items, including active pixels, selection size, palette settings, data limits and zone lines, all of which can be copied and pasted between images or pasted to all images. Selection size and zones lines can also be cut from the current image. The difference between copy/paste options and copy/paste internally is that items copied internally are not placed on the clipboard and can therefore not be pasted outside FluorImager.



Copy to Clipboard.

Copy to the clipboard allows access to many of the main copy items that can be found under Edit → Copy to clipboard....

There are an extensive range of clipboard copy functions, see Edit → Copy to clipboard... for more details.



Fluorescence Terminology

[A](#) [B](#) [C](#) [D](#) [E](#) [F](#) [G](#) [H](#) [I](#) [J](#) [K](#) [L](#) [M](#) [N](#) [O](#) [P](#) [Q](#) [R](#) [S](#) [T](#) [U](#) [V](#) [W](#) [XYZ](#) ...

A

B

C

D

Dirac constant

The Dirac constant (h -bar) is equal to the [Plank constant](#) divided by 2π ($h/2\pi$). The value of h -bar is 1.054×10^{-34} Js.

DeltaF (ΔF)

This term refers to the difference in fluorescence signal at two defined points. One of the many ways in which it has been used is to define the difference between F' measured immediately before a saturating pulse and Fm' measured at the peak of the same saturating pulse. Within the FluorImager program and these help files Fq' is used in this context.

E

ETR

Electron transfer rate.

F

F'

The fluorescence signal at any point between Fo' and Fm' . Within the FluorImager program and these help files, F' is used in preference to F_s and F_t , which have often been used in the same context.

Fm

The maximum fluorescence signal (when all PSII centres are in the [closed state](#)) from dark-adapted material.

Fm'

The maximum fluorescence signal (when all PSII centres are in the [closed state](#)) from light-adapted material.

$Fm/Fm' - 1$

A way of quantifying changes in the level of [non-photochemical quenching](#) of chlorophyll fluorescence ([NPQ](#)).

F_o

The minimum fluorescence signal (when all PSII centres are in the [open state](#)) from dark-adapted material.

 F_o'

The minimum fluorescence signal (when all PSII centres are in the [open state](#)) from light-adapted material. Within *FluorImager*, virtual F_o' images (which are required for the construction of [Fv/Fm'](#) and [Fq'/Fv'](#) images) are constructed using the [F_o' calculation method](#).

 F_{PSI}

The contribution of PSI fluorescence to the total fluorescence signal. Expressed as a percentage of the total fluorescence signal at [F_o](#) or [F_o'](#). See [PSI fluorescence dialog](#).

 F_q'

The difference between [F'](#) measured immediately before a saturating pulse and F_m' measured at the peak of the same saturating pulse. The non-specific term [DeltaF](#) (ΔF) has often been used in the same context.

 F_s

In most situations, this term is equivalent to [F'](#).

 F_t

In most situations, this term is equivalent to [F'](#).

 F_v

Variable fluorescence (difference between [F_o](#) and [F_m](#)) from dark-adapted material.

 F_v'

Variable fluorescence (difference between [F_o'](#) and [F_m'](#).) from light-adapted material

 F_v/F_m

Provides an estimate of the [PSII maximum efficiency](#) within dark-adapted material.

 F_v'/F_m'

Provides an estimate of the [PSII maximum efficiency](#) within light-adapted material.

 F_q'/F_m'

Provides an estimate of the [PSII operating efficiency](#) within light-adapted material.

 $F_v/(F_m.F_o)$

This term is equivalent to [1/F_o – 1/F_m](#). When normalised to the initial value, it provides an estimate of the change in the maximum PSII photochemical efficiency that can be normally be attributed to photoinactivation of PSII centres.

 F_q'/F_v'

A factor relating the [PSII maximum efficiency](#) and the [PSII operating efficiency](#). Mathematically, [F_q'/F_v'](#) is equivalent to [qP](#).

G

Genty factor

A term often used to describe the [PSII operating efficiency](#) (after [Genty, Briantais and Baker, 1989](#)).

H**I****Irradiance**

Refers to the 'energy flux density' of light, which has the units of $\text{J m}^{-2} \text{s}^{-1}$ or, more usually, W m^{-2} . See also; [PAR](#), [PPFD](#) and [photon irradiance](#).

J**K****Kautsky curve****L****M****N****Non-photochemical quenching**

Quenching of [variable fluorescence](#) that can be attributed to processes other than photochemistry.

Non-radiative decay

In the context of PSII photochemistry, non-radiative decay usually refers to the transition of an electron in the outermost shell of a chlorophyll *a* molecule from the first excited state to the ground state that results in an increase in the overall kinetic energy of the molecule, rather than the emission of a photon as fluorescence or a charge separation event.

NPQ

The term NPQ is widely used to represent [non-photochemical quenching](#). It is most frequently associated with the fluorescence parameter $(F_m / F_m') - 1$, which is a rearrangement of the [Stern-Volmer equation](#) (Bilger and Björkman, 1990). This equation simply states that the reciprocal of fluorescence yield is proportional to quencher concentration. So, for example, an increase in $(F_m / F_m') - 1$ from 1 to 2 could be interpreted as a doubling of quencher concentration. In reality, there is little evidence that fluorescence yield is actually modulated by quencher concentration and it should be appreciated that a change in the rate constant of a quenching mechanism, at any location within the PSII pigment bed, would be indistinguishable from a change in quencher concentration. Although $(F_m / F_m') - 1$ can clearly be a useful parameter, it is important to recognize that it is simply monitoring the rate constant for [non-radiative decay](#), normalized to the dark-adapted level. Consequently, it is only valid to compare

values among samples if it is known that the samples started off in the same condition: very similar initial values of [Fv/Fm](#) would be a good indication of this.

O

Open and closed PSII and PSI centres

Reaction centres are defined as being open if they are capable of stable charge separation (photochemistry) and closed if they are not. In the case of PSII, stable charge-separation results in oxidation of P680 and reduction of Q_A . Since the oxidation of Q_A^- is orders of magnitude slower than reduction of $P680^+$, a PSII centre is defined as being open when Q_A is in the ground state and closed when Q_A is reduced. Conversely, a PSI centre is open when P700 is in the ground state and normally closed when P700 is oxidised ($P700^+$).

P

PET

Photosynthetic electron transfer.

Photon irradiance

Photon irradiance is a term that is equivalent to [PPFD](#) and is preferred by some phycology journals. See also; [PAR](#), [PPFD](#) and [irradiance](#).

Planck constant

Formerly Planck's constant. The energy of a single photon (ϵ [epsilon]) can be related to it's frequency (ν [nu]) through the 'Planck constant' (h) such that:

$$\epsilon = h\nu$$

The value of h is 6.626×10^{-34} Js. See also [Dirac constant](#).

PPFD (photosynthetically active photon flux density)

PPFD is the term preferred by most plant journals. The usual units are $\mu\text{mol m}^{-2} \text{s}^{-1}$. Please note that PPFD is NOT interchangeable with [irradiance](#) or [PAR](#). Some phycology journals prefer the term [photon irradiance](#).

PAR (photosynthetically active radiation)

Although it is not incorrect to use PAR to refer to the photon content of light, it should be noted that "... a PAR of $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ " (for example) is incorrect because it doesn't include a reference to what is being measured. The correct statement would be "... a PAR of $250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ". See also [PPFD](#), [irradiance](#) and [photon irradiance](#).

PSII maximum efficiency

The efficiency with which light absorbed by the pigment matrix associated with PSII is used to drive stable photochemistry when all PSII centres are in the [open state](#). An estimate of the PSII maximum efficiency is provided by [Fv/Fm](#) in the dark-adapted state and [Fv/Fm'](#) in the light-adapted state.

PSII operating efficiency

The efficiency with which light absorbed by the light-harvesting system associated with PSII is used to drive stable photochemistry, in the light-adapted state. Because a fraction

of PSII centres are closed in the light-adapted state, the PSII operating efficiency is lower than the PSII maximum efficiency. Its value can be estimated through F_q'/F_m' , which is equivalent to the so-called '[Genty factor](#)'.

PSII photochemical factor

A factor relating the [PSII maximum efficiency](#) and [PSII operating efficiency](#), which provides an estimate of the fraction of the [PSII maximum efficiency](#) that is actually realised.

Q

Quenching coefficients

The system of quenching coefficients includes one term (qP) that is related to photochemical quenching. All of the remaining quenching coefficients relate to [non-photochemical quenching](#) processes: qN is the coefficient of total non-photochemical quenching; qE , the coefficient of energy-dependent quenching; qT , the coefficient of [non-photochemical quenching](#) associated with state-transitional changes and; qI , the coefficient of non-photochemical quenching associated with photoinhibition. They are usually calculated as:

$$1 - \frac{F_v'}{F_v}$$

Where F_v is the variable fluorescence in the initial (dark-adapted) state and F_v' is the variable fluorescence at the point at which the value of a particular coefficient is being calculated. A significant problem with this method is that any change in the effective rate constant for PSII photochemistry will decrease the value of the supposedly [non-photochemical quenching](#) coefficient that is being calculated. An example of where this might occur is when a healthy plant is subjected photoinhibitory conditions (e.g. a combination of high light and low temperature). Under photoinhibitory conditions, the rate at which PSII centres are inactivated exceeds the rate at which they are replaced, with a consequent decrease in the effective rate constant for PSII photochemistry.

qN

One of two widely used [quenching coefficients](#) (the other being qP), the so-called 'co-efficient of [non-photochemical quenching](#)' is usually calculated as the change in variable fluorescence between the dark- and light-adapted states. This parameter is not calculated by FluorImager. [NPQ](#) provides similar information.

qP

So-called 'co-efficient of photochemical quenching'. Mathematically, qP is equivalent to F_q'/F_v' ([PSII photochemical factor](#)). It has frequently been used (incorrectly) to estimate the fraction of PSII centres in the open state.

R

S

Stern-Volmer quenching

A convenient way of describing the relationship between chlorophyll fluorescence yield and downregulation is to treat downregulation as a Stern-Volmer quenching process. The

Stern-Volmer equation simply states that the reciprocal of fluorescence yield is proportional to quencher concentration.

$$\Phi_F \propto k_A \cdot [A] + k_B \cdot [B] + \dots$$

Where:

Φ_F is the yield of chlorophyll fluorescence, A and B are Stern-Volmer quenchers and k_A and k_B are the rate constants for the quenching of the singlet excited state of chlorophyll by A and B , respectively. See [NPQ](#) for further information.

T

U

V

Variable fluorescence

The difference between [Fo](#) and [Fm](#) or [Fo'](#) and [Fm'](#). Termed F_v in the dark-adapted state, F_v' in the light-adapted state. Variable fluorescence is modulated by both photochemical and [non-photochemical quenching](#) processes.

W

XYZ

...

1/Fo - 1/Fm

Originally described by Havaux et al. (1991). This parameter is equivalent (mathematically and conceptually) to [Fv/\(Fm.Fo\)](#), which was originally described by Baker and Dominy (1980).

1 - Fv'/Fm'

This parameter originates from a study by Demmig-Adams et al. (1986), in which it was used (incorrectly) to estimate the yield of [non-radiative decay](#) processes at PSII, in the light adapted state. This parameter actually provides an estimate of what the combined yield of [non-radiative decay](#) processes and chlorophyll fluorescence would be, if all PSII centres were in the open state at the point of measurement. Since the fraction of PSII centres in the open state tends to decrease with increasing incident PPFD, this parameter becomes an increasingly inaccurate method of estimating the yield of [non-radiative decay](#) processes at PSII.

FluorImager Software Terminology

[A](#) [B](#) [C](#) [D](#) [E](#) [F](#) [G](#) [H](#) [I](#) [J](#) [K](#) [L](#) [M](#) [N](#) [O](#) [P](#) [Q](#) [R](#) [S](#) [T](#) [U](#) [V](#) [W](#) [XYZ](#) ...

A

Active pixels

The active pixel locations within new images are defined using the [map image](#): If a pixel location contains a zero value within the map image, the same location in the new image will also be zero. When trace data points are constructed, pixel locations are excluded from the pixel count if they had a zero value within the map image or the data point image.

Administrator (of the FluorImager system)

Setting up a system for data acquisition or the running of either calibration routine requires a password. This password is known to the administrator (the person responsible for the instrument).

B

C

Colonies

Up to 250 colonies can be isolated within the [map image](#) using the Image(s)→Isolate colonies command. Mean values are calculated for each colony within raw fluorescence images and parameter images. Colonies can be merged by isolating the required colonies from the rest of the image and then selecting Image(s)→Merge colonies. A single colony can be split into two or more by using the tools within the [Modify image dialog box](#). These procedures should be performed in the following order: i) Run the Image(s)→Isolate colonies command; ii) split colonies; iii) re-run the Image(s)→Isolate colonies command; iv) merge colonies.

Current F_m

All of the fluorescence parameters calculated within FluorImager require a value for [Fm](#) or [Fm'](#) (with the exception of the parameters calculated from fluorescence transients). Some parameters that require a value for [Fm'](#) also require a value for [Fm](#) and, in some cases, [Fo](#). Specifically, [Fv/\(Fm.Fo\)](#), [Fv'/Fm'](#) and [Fq'/Fv'](#) all require values for [Fm](#) and [Fo](#) while [NPQ](#) requires a value for [Fm](#). The Current F_m is used in the calculation of these parameters. See Select the [current \$F_m\$](#) for details.

Current image

The large image in the middle of the FluorImager program screen (the currently selected image).

Current pulse

This is the [saturation pulse](#) that is currently selected from within the main trace area.

Current transient

This is the [transient](#) that is currently selected from within the main trace area. A saturation pulse or a transient can be selected from within the main trace area. Consequently, there can be a [current pulse](#) or a current transient, but not both.

D

E

F

Fo' calculation method

In order to calculate [Fv'/Fm'](#) and [Fq'/Fv'](#), the value of [Fo'](#) must be known. A widely used method for the estimation of [Fo'](#) is to expose the sample to weak far-red light in the absence of actinic light. It is assumed that, under these conditions, Q_A is maximally oxidized due to preferential excitement of PSI (relative to PSII) by the far-red light. There are at least two potential sources of error with this method. Firstly, it is very likely that [non-photochemical quenching](#) will partly reverse during the far-red light treatment. Secondly, and more importantly, this method relies on the complete oxidation of Q_A (which, in turn, relies upon oxidation of the plastoquinone pool) within a relatively short time (typically, 3 s), in the presence of a ΔpH across the thylakoid membrane. It is almost certainly the case that, under these conditions, the oxidation of plastoquinone at the Q_O site of the cytochrome b₆f complex imposes a much greater limitation to non-cyclic electron flow than does the rate of excitation of PSI (Bendall, 1982). Consequently, it is unlikely that the preferential excitation of PS I by far-red light treatment will have the desired effect and that the level of [Fo'](#) will be overestimated, particularly at high PPFD.

The FluorImager program employs an alternative method of [Fo'](#) estimation (Oxborough and Baker, 1997). With this method, a virtual [Fo'](#) image is created during the construction of [Fv'/Fm'](#) and [Fq'/Fv'](#) images. This image is created using the [Fm'](#) selected for construction of the [Fv'/Fm'](#) and [Fq'/Fv'](#) images, plus suitable [Fo](#) and [Fm](#) images. If there are multiple [Fo](#) and [Fm](#) images within a file, the user can select the one's that are to be used in the construction of the virtual [Fo'](#) image.

$$Fo' = \frac{Fo}{Fv/Fm + Fo/Fm'}$$

It has been suggested that this method should not be used to determine [Fo'](#) in situations where plants are stressed and significant amounts of photoinhibition have occurred (Maxwell and Johnson, 2000). In reality, the only requirements for the calculation of [Fo'](#) from the above equation are that (i) all the PSII centres are open at the point at which [Fo](#) is measured, (ii) there is no reversal of down-regulation between the measurement of [Fo](#) and [Fm](#), and (iii) there is no reversal of photoinhibition between the measurement of [Fm'](#) and [Fm](#). In most situations, these requirements are very easily met. Given the inherent difficulty of accurately measuring [Fo'](#), it can be argued that the calculation of [Fo'](#) gives a more reliable estimate of this fluorescence level.

G

H

I

J**K****L****M****Map image**

A map image is normally taken at the start of every experiment. This step is important for two reasons:

Firstly, the maximum pixel values within this image are used to set the optimum camera gain for all subsequent images, including the images that are used in the construction of the continuous fluorescence trace. If the signal is too high when the map image is taken, the user is informed and asked to decrease the amount of light reaching the CCD: this is normally done by closing down the aperture, although neutral density filters can also be used.

Secondly, The map image defines all the active (non-zero) pixel locations for new images. If a pixel location within the map image is zero, the same pixel location within all new images will also be zero, even if it is greater than zero in the new image. Pixels with a value of zero are not included in the calculation of mean values. Non-zero pixels can be deleted (made zero) from the map image using the tools built into the Modify image dialog.

Modal and modeless dialogs

Modal dialogs must be closed before the user can interact with the rest of the program. Modeless dialogs can be left open while the user interacts with other parts of the program.

N**O****P****Parameter images**

The parameter image types constructed within FluorImager are; [\$F_v/F_m\$](#) , [\$F_v/\(F_m.F_o\)\$](#) , [\$F_q/F_m'\$](#) , [\$F_v/F_m'\$](#) , [\$F_q/F_v'\$](#) , and [NPQ](#).

Protocol

Protocols represent the highest level of automation within the FluorImager program. They are constructed through the Protocol dialog.

Q**R**

Raw images

This term is applied to the [F_o](#), [F_m](#), [F'](#) and [F_m'](#) images that are used in construction of the [parameter images](#).

Region image

A region image defines a sub-set of the active pixels within the [map image](#). A separate fluorescence trace is recorded for each region image (up to 14 region images can be created within a file, in addition to the map image). These traces can be shown/hidden by selecting View region trace from a popup menu that is launched when the right mouse button is clicked while the cursor is over the [thumbnail](#) of the region image in question. See Working with region images for further details.

S**Saturation pulse**

A short pulse of light (typically, a [PPFD](#) of a few thousand $\mu\text{mol m}^{-2} \text{s}^{-1}$) that is used to measure [F_m](#) and [F_m'](#). Within FluorImager, individual PPFDs and saturation pulse lengths can be set at each of the 'stock' incident PPFD levels, through the Light settings dialog.

Settings files (*.set)

When the program is started, the default settings are loaded for the person who is logged on. If different settings are loaded by opening a settings file, these settings become the default and are loaded the next time the program is started by the same user. Settings files can be loaded and saved through Settings → .set files →.

T**Thumbnail (image)**

Thumbnail versions of all the images within a file are shown in the [thumbnail images](#) area.

Transient

In the context of FluorImager, a transient is the increase in fluorescence that results from the application of sub-saturating incident light to dark-adapted material (the initial part of a Kautsky curve). FluorImager has the facility for the setting up of 6 stock transients, which can be applied at any time that a fluorescence trace is being recorded. These transients can be set up through the [Transient settings dialog](#). A transient can also be applied as part of a [protocol](#).

U**V****W****Width and Skew (of a colour palette)**

The width and skew controls provide a high degree of control over the presentation of false colour images. The width of a colour palette determines the dominance of the colours at either end of the palette range. Its value can be set anywhere between 0 and

100. The skew control moves the central region of the palette to the left or right of centre. It's value can be anywhere between -50 (left) and +50 (right).

XYZ

Zone line

Zone lines can be used to divide an image into a maximum of 96 zones. This method of dividing an image is particularly useful when working with well plates and other well ordered samples. When working with randomly distributed samples, the [colonies](#) or [region images](#) methods may be more suitable. See 'Work with zone lines (96 well plate example)' for more information.

Zooming

When an area is selected within the [current image](#), the selected area is magnified by the highest possible integer value (while keeping the selection within the boundaries of the [current image](#) area of the program screen or [Modify image dialog](#)). This magnification can be switched off and on by clicking the right mouse button within the selected area of the image and selecting Zoom on/off from the popup menu or by selecting Image(s) → Zoom on/off from the menu bar.

Appendix 1.

Lights and Camera Calibration.

The lighting and camera calibrations do not need altering unless the light units or camera have been replaced

Lighting calibration.



The camera must be connected to perform this calibration. Left click Settings on the top toolbar, left click Calibration at the bottom of the menu, left click Run Lights Calibration. Using a calibrated light meter, such as the Li-Cor LI-250 positioned in the centre of the sample stage, enter recorded values in the initially blue highlighted check boxes. The sample stage must be lowered from its standard position, 140mm above the instrument base, by

the height of the light meter sensor. In the case of the above meter this is 25mm. When all nine check boxes have been carefully completed, Save and exit.

Camera Calibration



To perform this calibration, which must be made if the lights calibration has been changed, follow the on – screen instructions. The calibration takes several minutes and no settings should be changed whilst the calibration is in progress. You will be instructed to remove the infra –red filter in the telescopic lens tube. This is held by friction to the lens, to remove the filter, raise the camera rack and pinion stage from its normal position, both zeros opposite one another, to the top of its travel and lock into position using the clamp at the rear of the stage. Firmly pull down the narrower tube with a slight rocking motion and store the filter safely, avoid touching it.

Place a non reflective black cloth on top of the sample stage and ensure the sample stage is at its normal height 140mm from base of instrument. Press the Connect camera button on the toolbar bar. Left click Settings on the top toolbar, left click Calibration at the bottom of the menu, left click Run camera calibration. Before you accept yes ensure the instrument door is shut, it must not be opened during the calibration. An on-screen message will tell you when calibration is complete. Remove the black cloth and restore the sample stage to preferred height

Lighting Alignment.

The 16 light 'bricks' are factory set for even illumination over the sample stage. If they have been accidentally disturbed or realigned for optimum illumination over irregular samples they will have to be realigned again. With the continuous (actinic) light on at a PPFD of a few hundred $\mu\text{mol m}^{-2} \text{s}^{-1}$ a light meter located at the correct height, as in the above section, Lighting Calibration, should be moved about over the sample stage and recorded light levels noted.

As a starting point, arrange the LED panels in the following way:

- a) The six central panels should be horizontal.
- b) The eight corners panels should be adjusted in pairs, to give the maximum signal close to the adjacent corner.
- c) The remaining two panels and the six central panels should then be adjusted to even the light out.

Please note that:

- Even small movements of the central six panels can have quite large effects
- It is normal to end up with one or more of the central bricks pointing slightly away from the centre

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